Summary

1. Mono-esterification of dibasic acid anhydrides with primary mono- and polyhydric alcohols proceeds instantaneously and quantitatively at room temperature.

2. Even high molecular weight acid anhydrides, for example, rosin–maleic anhydride adduct, proceed readily to mono-esterification.

3. This work serves as the basis for the analytical determination method for dibasic acid anhydrides in a mixture with their acids. It can also serve to determine the rate of rearrangement of fumaric acid to maleic anhydride.

ELIZABETH, NEW JERSEY RECEIVED JANUARY 21, 1947

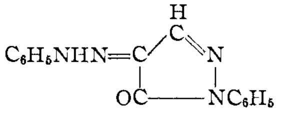
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

The Action of Periodic Acid on Glucose Phenylosazone

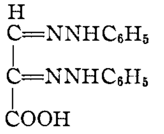
BY ERWIN CHARGAFF AND BORIS MAGASANIK

In the course of studies on the enzymatic dehydrogenation of stereoisomers of the inositol group,[1] which included the use of periodic acid as an analytical tool, it became desirable to examine the action of this oxidant on a phenylosazone of known structure, *viz.*, glucosazone.

The oxidation of this osazone by periodic acid in boiling 50% alcohol had already been studied some time ago.[2] Under the rather vigorous conditions used it had been shown to lead to the formation of 1-phenyl-4-phenylhydrazonopyrazolone-5 (I).[3] This compound was assumed to have been formed by dehydration from the original oxidation product, the 1,2-bisphenylhydrazone of mesoxalic acid semialdehyde (II).[4] But the production of II can hardly be reconciled with what is known at present of the behavior of periodic acid.[5] The oxidative cleavage of glucosazone by periodic acid was therefore studied in greater detail by procedures that had proved satisfactory in previous work in this Laboratory.[6]



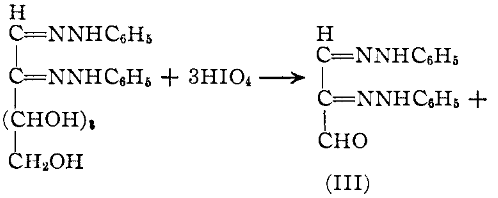
(I)



$$\begin{array}{l}\mathrm{H}\\ \mathrm{C{=}NNHC_6H_5}\\ |\\ \mathrm{C{=}NNHC_6H_5}\\ |\\ \mathrm{COOH}\end{array}$$

(II)

When glucose phenylosazone in 66% ethanol was treated with periodic acid *at room temperature* three moles of oxidant were consumed per mole of osazone and a compound was isolated (yield 85%) whose analytical composition showed it to be the hitherto undescribed *1,2-bisphenylhydrazone of mesoxalaldehyde* (III). Under the experimental conditions employed here the oxidative action of periodic acid appeared, therefore, to proceed in the expected fashion.

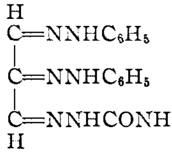


$$\begin{array}{l}\mathrm{H}\\ \mathrm{C{=}NNHC_6H_5}\\ |\\ \mathrm{C{=}NNHC_6H_5} + 3\mathrm{HIO_4}\\ |\\ \mathrm{(CHOH)_3}\\ |\\ \mathrm{CH_2OH}\end{array} \longrightarrow \begin{array}{l}\mathrm{H}\\ \mathrm{C{=}NNHC_6H_5}\\ |\\ \mathrm{C{=}NNHC_6H_5} +\\ |\\ \mathrm{CHO}\end{array}$$

(III)

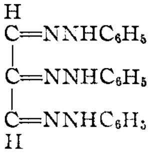
$$2\mathrm{HCOOH} + \mathrm{HCHO} + \mathrm{H_2O} + 3\mathrm{HIO_3}$$

Compound III was further characterized by its conversion to the corresponding semicarbazone (IV) and to the known trisphenylhydrazone[7] (V).



$$\begin{array}{l}\mathrm{H}\\ \mathrm{C{=}NNHC_6H_5}\\ |\\ \mathrm{C{=}NNHC_6H_5}\\ |\\ \mathrm{C{=}NNHCONH_2}\\ \mathrm{H}\end{array}$$

(IV)



$$\begin{array}{l}\mathrm{H}\\ \mathrm{C{=}NNHC_6H_5}\\ |\\ \mathrm{C{=}NNHC_6H_5}\\ |\\ \mathrm{C{=}NNHC_6H_5}\\ \mathrm{H}\end{array}$$

(V)

The quantitative comparison of the experimental conditions leading, at room temperature, to the formation of the aldehyde III and at an elevated temperature to that of the pyrazolone I gave interesting results. In both cases three moles of periodic acid were consumed; but the titration of the total available oxidizing capacity (*i. e.*, of the sum of excess periodic and iodic acids in the reaction mixtures) with sodium thiosulfate in acidic solution indicated that in boiling alcohol one additional gram-atom of oxygen, derived from the iodic acid, had been used. The conclusion appeared justified that the aldehyde III, formed as the primary oxidation product in both sets of conditions, was further oxidized by the boiling iodic acid solution.

It remained to be shown whether the cyclization to the pyrazolone I proceeded *via* the acid II. When the aldehyde III was heated with iodic acid in 50% ethanol it was converted to I. That the production of the pyrazolone was not due to the dehydration, under acidic conditions, of II, formed as the primary oxidation product, could be shown in experiments in which the acid II was subjected to a similar treatment with 1 *N* hydrochloric acid in 50% ethanol. Under these conditions the unchanged compound II was recovered.

Even more convincing was the direct formation

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[1] E. Chargaff and B. Magasanik, *J. Biol. Chem.*, **165**, 379 (1946).

[2] P. Karrer and K. Pfaehler, *Helv. Chim. Acta*, **17**, 766 (1934).

[3] L. Knorr, *Ber.*, **21**, 1201 (1888).

[4] O. Nastvogel, *Ann.*, **248**, 85 (1888).

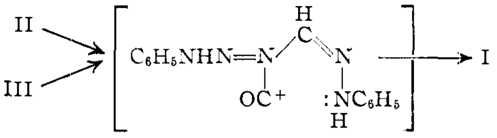
[5] E. L. Jackson in R. Adams, "Organic Reactions," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 341.

[6] D. B. Sprinson and E. Chargaff, *J. Biol. Chem.*, **164**, 433 (1946).

[7] H. v. Pechmann and K. Jenisch, *Ber.*, **24**, 3255 (1891).

of the pyrazolone I by the action of silver oxide in alkaline solution on the aldehyde III. Compound II can be dissolved in hot sodium carbonate solution and recovered unchanged by acidification.[3] All these facts appear to exclude the function of II as an intermediate in the conversion of III to I. The acid II can, however, under different conditions give rise to I, *viz.*, by prolonged treatment with alcoholic hydrochloric acid[8] or with acetic anhydride.[3] The latter reaction was confirmed in the course of this work.

The results outlined here are compatible with the following scheme for the conversion of II and III, respectively, to I. In both cases, the reaction



may be assumed to proceed through an intermediate formed by the action of strong anhydrous acid on II, removing the hydroxyl from the carboxyl group, or by that of oxidizing agents on III, resulting in the detachment from the aldehyde group of the hydrogen ion and two electrons. Nitrogen atom 1 with an unshared pair of electrons now is in a favorable position for ring closure; and thus, with the removal of hydrogen ion from nitrogen atom 1, the pyrazolone I is formed.

The absorption spectra of compounds I, II and III are shown in Fig. 1. The spectra of II and III are not dissimilar, with the exception of the absorption peak of II at 273 and of III at 314 mμ. The spectrum of I, however, is quite different, thus emphasizing again the distinction between the cyclic pyrazolone I and the open chain compounds II and III.

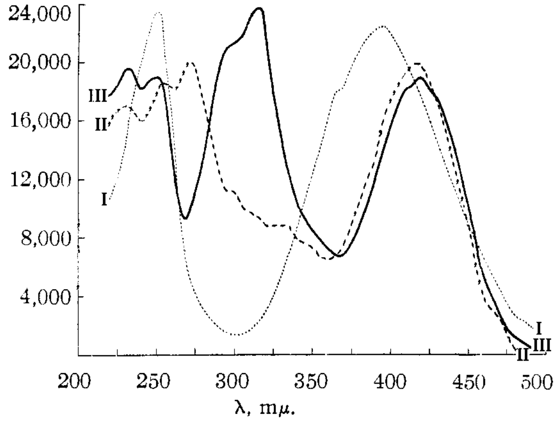


Fig. 1.—Absorption spectra in absolute ethanol of compounds I, II and III.

The absorption spectra of the 1,2-bisphenylhydrazones of the two representatives of lower oxidation states corresponding to the three-carbon system studied here, *viz.*, the derivatives of pyruvaldehyde and hydroxypyruvaldehyde, have been reported before.[9] Their visual absorption peaks were found at 361 and 381 mμ, respectively, *i. e.*, at shorter wave lengths than in the aldehyde III and the acid II.

Experimental

1,2-Bisphenylhydrazone of Mesoxalaldehyde (III).—A solution of 5.4 g. of glucose phenylosazone (15 millimoles) in two liters of warm 66% ethyl alcohol was cooled to room temperature and treated with 10 g. of paraperiodic acid (44 millimoles) in 70 cc. of water. The yellow-orange precipitate which appeared at once was collected, following dilution of the mixture with 500 cc. of water, washed with 66% alcohol and dried *in vacuo*. Two recrystallizations of this material (3.4 g., 85% of the theoretical yield) from 66% alcohol yielded yellow-orange needles melting[10] (with decomposition) at 198°. The substance was very insoluble in water, acid, alkali, and only slightly soluble in the organic solvents tested. It gave a positive Schiff test only after several hours of contact with the reagent.

Anal. Calcd. for $C_{15}H_{14}ON_4$: C, 67.6; H, 5.3; N, 21.0. Found: C, 67.4; H, 5.1; N (Dumas), 21.0.

1,2-bis-Phenylhydrazone-3-semicarbazone of Mesoxalaldehyde (IV).—A mixture of 170 mg. of III, 76 mg. of semicarbazide hydrochloride in a few drops of water and 68 mg. of potassium acetate was heated to boiling with two cc. of alcohol. The hot solution was diluted with hot water to beginning turbidity and cooled, when a light yellow substance separated (weight 190 mg., 92% yield). Three recrystallizations from benzene-alcohol yielded the semicarbazone as light yellow needles melting (with decomposition) at 204–205°, insoluble in water and benzene, soluble in alcohol.

Anal. Calcd. for $C_{16}H_{17}ON_7$ (dried at 110° *in vacuo*): C, 59.4; H, 5.3; N, 30.3. Found: C, 59.7; H, 5.4; N, 29.8.

1,2,3-tris-Phenylhydrazone of Mesoxalaldehyde (V).—A suspension of 50 mg. of III in one cc. of ethanol was warmed with 0.2 cc. of phenylhydrazine until all was dissolved. The solution, diluted with 5 cc. of alcohol, was heated to 60° and the tris-phenylhydrazone precipitated by the addition of 20 cc. of hot water. The material, collected after chilling, washed with 20% alcohol, and dried, weighed 64 mg. (yield 95%). It was recrystallized twice from 60% alcohol: light yellow small plates, melting at 166–167°, insoluble in water, acid, alkali, easily soluble in alcohol and ether.[11]

Anal. Calcd. for $C_{21}H_{20}N_6$: C, 70.8; H, 5.7; N, 23.6. Found: C, 70.5; H, 5.5; N, 22.8.

Quantitative Measurement of Oxidant Consumption. (1) **At Room Temperature.**—Twenty-cc. portions of solutions of between 31 and 76 micromoles of glucose phenylosazone in 66% alcohol were treated with 325 micromoles of paraperiodic acid in 0.5 cc. of water at room temperature for one hour. The mixtures, in which a yellow precipitate appeared almost at once, were diluted with 30 cc. of water. The excess periodic acid was determined with 0.1 *N* sodium arsenite by titration in sodium bicarbonate solution of the iodine liberated after addition of potassium iodide. The residual total oxidizing capacity was estimated in acidic solution with 0.1 *N* sodium thiosulfate. The consumption (per mole of glucosazone) of periodic acid, determined in several individual experiments, corresponded to 2.93, 3.13, 2.96 moles, that of the total available oxygen to 3.0, 3.1 gram-atoms of oxygen.

(2) **In Boiling Alcohol.**—Several samples, corresponding to 51–75 micromoles, of glucosazone, were suspended

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(8) W. Will, *Ber.*, **24**, 3831 (1891).

(9) L. L. Engel, This Journal, **57**, 2419 (1935).

(10) The melting points, reported without correction, were determined with an electrically heated stage (Fisher–Johns).

(11) v. Pechmann and Jenisch[7] report m. p. 166°.

in 12 cc. of 66% alcohol and heated to boiling in the presence of 325 micromoles of paraperiodic acid in 0.5 cc. of water. The red solutions were permitted to stand for one hour, and the unused periodic acid as well as the sum of iodic and periodic acids were determined as described before. In these determinations 3.07 and 2.96 moles of periodic acid were consumed by one mole of glucosazone; but the values found in two experiments for the total oxidizing capacity showed that 4.1 gram-atoms of oxygen had been used.

Oxidation of the 1,2-bis-Phenylhydrazone of Mesoxalaldehyde (III).—When 940 mg. of III was boiled for five minutes with 200 cc. of 50% ethanol containing 1.85 g. of iodic acid, a dark red-brown solution resulted which on being cooled deposited 310 mg. of the crude oxidation product (yield 33%). This material was purified by precipitation with acid from its solution in hot 0.2 *M* sodium carbonate and then by three recrystallizations from ethanol. The *pyrazolone* I was obtained as small orange needles melting at 150°, soluble in alkali, slightly soluble in alcohol and chloroform, insoluble in water and acid.

In another experiment, 1.85 g. of silver nitrate in 200 cc. of water were added to a suspension of 1.33 g. of the aldehyde III in 150 cc. of ethanol. To this mixture 16.5 cc. of *N* potassium hydroxide was added dropwise with constant stirring in the course of one hour.[12] An undissolved fraction, from which a large proportion of the unchanged aldehyde III could be recovered, was filtered off and the filtrate acidified when 160 mg. of the crude *pyrazolone* I separated. Three recrystallizations from alcohol and chloroform brought the melting point to 149–150°.

For purposes of comparison 1-phenyl-4-phenylhydrazonopyrazolone-5 (I) also was prepared according to Knorr[3] by treating the 1,2-bisphenylhydrazone of mesoxalic acid semialdehyde (II) with acetic anhydride. The starting material had been prepared by Dr. D. B. Sprinson from hydroxypyruvic acid by the method recently published from this Laboratory.[13] The pyrazolone melted at 149–150° and showed no depression of the melting point when mixed with preparations obtained by the oxidation of the aldehyde III.

Absorption Spectra.—The spectra of compounds I, II and III, reproduced in Fig. 1, were determined with a Beckman photoelectric quartz spectrophotometer. About 0.023 millimolar solutions in absolute ethyl alcohol were employed. We are indebted to Dr. E. Brand and Miss M. Crymble of this Department for help with these measurements.

Acknowledgments.—The authors wish to thank Mr. W. Saschek for the microanalyses.

Summary

Glucose phenylosazone, oxidized at room temperature with periodic acid, gave rise to the expected aldehyde, the 1,2-bis-phenylhydrazone of mesoxalaldehyde (III) which was further characterized by conversion to its semicarbazone (IV) and the corresponding tris-phenylhydrazone (V).

At elevated temperatures the aldehyde III was further oxidized by the iodic acid present to 1-phenyl-4-phenylhydrazonopyrazolone-5 (I) which can also be produced by the action of dehydrating agents on the 1,2-bis-phenylhydrazone of mesoxalic acid semialdehyde (II). The probable mechanism of these transformations is discussed briefly.

NEW YORK, N. Y.

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[12] M. Delépine and P. Bonnet, *Compt. rend. acad. sci.*, **149**, 39 (1909).

[13] D. B. Sprinson and E. Chargaff, *J. Biol. Chem.*, **164**, 417 (1946).

[CONTRIBUTION FROM THE CHEMISTRY LABORATORY, NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

The Action of Copper Sulfate on the Phenylosazones of the Sugars. V. The Phenylosotriazoles of L-Rhamnose, L-Fucose and Melibiose

BY W. T. HASKINS, RAYMOND M. HANN AND C. S. HUDSON

This paper is a continuation of the articles previously published[1] describing the conversion of the sugar phenylosazones to the corresponding phenylosotriazoles through the action of copper sulfate. The phenylosotriazole from L-rhamnose (6-desoxy-L-*arabo*-hexose phenylosotriazole) is only moderately soluble in water at ordinary temperature and crystallizes readily from concentrated aqueous solutions, while the phenylosotriazoles from L-fucose and melibiose (6-desoxy-L-*lyxo*-hexose phenylosotriazole and 6-α-D-galactopyranosyl-D-*arabo*-hexose phenylosotriazole) are very soluble in water and crystallize best from non-aqueous solvents. All three phenylosotriazoles have sharp melting points and solutions of them do not mutarotate, in common with the properties of the other sugar phenylosotriazoles that were described in the previous articles.

The structures of the L-rhamnose and L-fucose phenylosotriazoles were proven to be those shown in the formulas by oxidation with sodium metaperiodate which in each case gave high yields of 2-phenyl-4-formyl-2,1,3-triazole together with the appropriate amounts of formic acid and acetaldehyde. The structure of melibiose phenylosotriazole was demonstrated by hydrolysis with dilute hydrochloric acid to produce D-glucose phenylosotriazole (D-*arabo*-hexose phenylosotriazole) and D-galactose in good yields.

We are indebted to Mr. Charles A. Kinser and Mrs. Betty Mount for the microchemical analyses.

Experimental

L-Rhamnose Phenylosotriazole.—To a suspension of 10 g. of L-rhamnose phenylosazone[2] in 900 ml. of boiling water was added a solution of 8.0 g. (1.1 molecular equivalents) of copper sulfate pentahydrate in 100 ml. of boiling water and the mixture was refluxed one hour. The cooled solution was filtered and on concentrating it *in vacuo* to 100 ml., spontaneous crystallization of the product took place; the crystals were dissolved by warming the mixture

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[1] Number IV was published in THIS JOURNAL, **69**, 1050 (1947).

[2] Fischer and Zach, *Ber.*, **45**, 3770 (1912).